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**Melissa K. Ha, Scott A. Schneider &
Lynn S. Adler**

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Facilitative pollinator sharing decreases with floral similarity in multiple systems

Melissa K. Ha¹ · Scott A. Schneider² · Lynn S. Adler³

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Abstract

Investigating the factors that determine whether interactions are competitive or facilitative is essential to understanding community structure and trait evolution. Co-flowering plants interact indirectly through shared pollinators, and meta-analyses suggest that phylogenetic relatedness and floral trait similarity may predict the outcome of these interactions. In a comparative approach, we manipulated the floral community across five focal species to assess how floral similarity and phylogenetic relatedness affect the outcome of interactions. To assess the extent of pollinator-mediated competition versus facilitation, we compared pollen limitation in five focal species growing with floral neighbors (either congeners or neighbors from a different family) relative to a control (growing alone). We measured floral morphology, color, and nectar traits to calculate multivariate floral similarity between species pairs and inferred a phylogeny to calculate phylogenetic distance. Pollinator-mediated interaction values were regressed against floral similarity and phylogenetic distance. We found evidence of pollinator-mediated facilitation in nine of 13 species pairs. Furthermore, floral similarity and phylogenetic distance reduced facilitative interactions, but the latter relationship was not significant when controlling for the identity of the focal species. Our results suggest that facilitative pollinator sharing is more common than reported in the literature, but co-flowering plant species with similar floral traits are less likely to facilitate pollination. A better understanding of the factors that promote facilitation versus competition has important potential applications for managing rare and invasive species.

Keywords Comparative study · Competition-relatedness hypothesis · Floral traits · Phylogenetic distance · Pollinator-mediated facilitation

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In this comparative experiment, the strongest pollinator-mediated facilitation occurred between plant species pairs with contrasting floral traits. This informs prediction of biotic interactions.

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✉ Melissa K. Ha
mha@yccd.edu

¹ Department of Biology and Ecology, Yuba College, 2088 North Beale Road, Marysville, CA 95901, USA

² Systematic Entomology Laboratory, USDA, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

³ Biology Department, 221 Morrill Science Center, University of Massachusetts, 611 North Pleasant Street, Amherst, MA 01003, USA

Introduction

Although interspecific competition has historically been emphasized in ecological studies (Lortie and Callaway 2009), the importance of facilitation in community structure and evolution is receiving increasing attention (Brooker et al. 2008; Brooker and Callaway 2009). Defined as biotic interactions in which one or both interacting species benefit, facilitation is now recognized as an important driver of ecological (McIntire and Fajardo 2014; Soliveres et al. 2015) and evolutionary processes (Soliveres et al. 2015). Facilitation may influence local establishment (Stachowicz 2001; Muhamed et al. 2013) and exert natural selection on facilitation-enhancing traits (Michalet et al. 2011) in a variety of organisms. Not only are the effects of facilitation far-reaching, but they can be distinct from those of competition. For example, both influence biodiversity, but competition drives exploitation of new niches while facilitation can create new niches (McIntire and Fajardo 2014). Specifically, facilitation

could ameliorate harsh environmental conditions, provide greater access to resources, or create a spatially complex habitat, allowing a greater variety of species to thrive in a region (McIntire and Fajardo 2014). Facilitation can also influence the effect of other factors on species diversity. A study of four habitat types found that regional species richness had a greater impact on local species richness in communities for which facilitation was the dominant interaction. In this case, facilitation may have increased the likelihood that a species from the regional pool could survive in a local community (Michalet et al. 2015). To better anticipate the differing ecological effects of facilitation versus competition, one must first classify biotic interactions as positive or negative. However, measuring the outcome of each pairwise biotic interaction in a community can be time-consuming or impractical. There is thus a need to identify the circumstances under which species interactions are facilitative versus competitive to predict the outcome of biotic interactions as well as their consequences (Stachowicz 2001).

Biotic interactions are context-dependent when the sign or magnitude of interactions varies with abiotic or biotic factors (Chamberlain et al. 2014). Identifying the conditions that determine the outcome of biotic interactions is important both for parameterizing models that predict coexistence (Cameron et al. 2009) and for designing appropriate habitat conservation measures for target species (Harrington et al. 2003). Two non-mutually-exclusive factors are often hypothesized to affect the outcome of biotic interactions: phylogenetic relatedness and trait similarity. The competition-relatedness hypothesis (Darwin 1859; Cahill Jr. et al. 2008) asserts that competition between species increases with their phylogenetic relatedness and has found support in many systems (Webb et al. 2006; Violle et al. 2011; Koepfel and Wu 2014), although there are several counterexamples (e.g., Cahill Jr. et al. 2008; Kunstler et al. 2012; Beaudrot et al. 2013). Trait overlap is a logical mediator of the relationship between phylogenetic relatedness and competition, and trait overlap can certainly increase competition (MacArthur 1958; Wagg et al. 2017). However, in some cases phylogenetic relatedness has been a stronger predictor of the strength or direction of biotic interactions than trait overlap (Violle et al. 2011) because all of the relevant traits mediating outcomes may not have been measured. Conversely, when closely related species do not resemble each other ecologically (Losos 2008; Bergamo et al. 2018), overlap of the relevant traits may be a better indicator of the outcome of biotic interactions than phylogenetic relatedness. Through assessing both phylogenetic relatedness and trait overlap, we can evaluate their relative contributions to determining the outcome of biotic interactions.

Most plant species are visited by generalist pollinators, and pollinator sharing among co-flowering plant species is common in nature (e.g. Stiers et al. 2014). The outcome

of pollinator sharing between plant species can range from competition, if floral neighbors decrease pollinator visitation or increase heterospecific pollen transfer (Dietzsch et al. 2011), to facilitation, if floral neighbors help attract (Johnson et al. 2003) or maintain pollinators (Moeller 2004). Trait overlap may influence whether plants compete for or facilitate pollination. Individual generalist pollinators can be constant to a specific flower type, selectively moving among flowers that are similar (Gegear and Laverly 2005). When different plant species have similar floral morphologies, constancy of generalist pollinators can increase heterospecific pollen transfer, decreasing reproductive success (Campbell and Motten 1985). Similarity in floral morphology may thus increase pollinator-mediated competition (Dietzsch et al. 2011; Muchhala et al. 2014), hereafter referred to as the competition-trait similarity hypothesis (Kunstler et al. 2012), but this does not occur all systems (Schemske 1981; Johnson et al. 2003). The competition-relatedness hypothesis may also apply to pollinator-mediated interactions; i.e., plant species may compete more when they are more closely related (Morales and Traveset 2009; Carvalheiro et al. 2014; Arceo-Gómez and Ashman 2016). However, indirect interactions between closely related species may be more likely to be positive when they are mediated by shared mutualists (Belt-rán et al. 2012). For example, pollinator visitation to *Clarkia xantiana* increased in the presence of congeners (Moeller 2004). Support for both hypotheses has varied across the literature; meta-analyses have supported both the competition-relatedness and competition-trait similarity hypotheses (Morales and Traveset 2009), only the competition-relatedness hypothesis (Carvalheiro et al. 2014; Arceo-Gómez and Ashman 2016), or neither hypothesis (Charlebois and Sargent 2017).

We applied a novel comparative approach to complement existing meta-analyses that examine the predictive value of phylogenetic relatedness and floral trait similarity. We manipulated the floral neighborhoods of multiple focal species in experimental arrays to represent a range of floral trait similarities and relatedness. A standardized measurement of pollen limitation determined whether pollinator-mediated interactions across the 13 species pairs in this field experiment were competitive or facilitative. We assessed the influence of phylogenetic relatedness on pollinator-mediated interactions and formally evaluated whether this influence is mediated by floral traits including morphology, color, and nectar production. We selected species that occur in western Massachusetts, USA, and paired them with species that varied in phylogenetic relatedness and floral similarity. In this way, we examined whether closely related plant species are likely to compete for pollinators (competition-relatedness hypothesis) and whether similarity in floral traits increases pollinator-mediated competition (competition-trait similarity hypothesis).

Materials and methods

Focal species selection

Selecting focal and neighboring species was an intensive process that considered feasibility of cultivation, co-flowering (i.e., blooming at the same time), natural occurrence in western Massachusetts, comparable plant sizes, and co-occurrence (i.e., documented to grow naturally in close proximity or to occur in similar habitats). When all criteria could not be met, the criteria were prioritized in the order listed. Co-occurrence was a lower priority because we were investigating whether phylogenetic or floral similarity could predict pollinator-mediated interactions rather than documenting the pollinator-mediated interactions of any particular plant community. Based on herbarium records, four of the 13 species pairs are known to co-occur (Table 1). It is possible or even likely that other species pairs co-occur but are not documented. We did not use pollinator sharing as an explicit criterion for species selection due to lack of information about pollinator identity, but our observations showed that most species were visited by generalists and so could share pollinators (Online Resource 1; see Supplemental Data with the online version of this article). Unrelated neighbors were randomly selected from a list of plants from a different family than the focal species that best met our criteria. This list was developed by referencing field guides, herbarium records, herbarium staff, and observations of plants in natural areas. Initially, our intent was to select a morphologically similar and dissimilar unrelated neighbor. However,

subsequent analysis of floral traits found no significant difference in floral similarity between these two groups compared to the focal species (Tukey pairwise comparisons on mixed-effects analysis of variance, $z = 0.81$, $df = 1$, $P = 0.7$). We thus treat floral similarity as a continuum rather than categorical factor, which better allows us to account for varying levels of similarity between each species pair. Floral similarity calculations are described in “Quantifying floral similarity and phylogenetic relatedness” below and Online Resource 2, which also describes plant sources and cultivation methods.

We examined pollinator-mediated interactions of five focal species: *Viola tricolor* L. (Violaceae), *Petunia axillaris* (Lam.) B.S.P. (Solanaceae), *Solanum dulcamara* L. (Solanaceae), *Myosotis scorpioides* L. (Boraginaceae), and *Lobelia inflata* L. (Campanulaceae). All but *L. inflata* occur in but are non-native to Western Massachusetts. Two focal species (*L. inflata* and *P. axillaris*) are annual, *V. tricolor* grows as an annual or perennial, and the other two species are perennial. *Viola tricolor* and *P. axillaris* occur in open fields, *S. dulcamara* occurs in open woods, and *L. inflata* occurs in either location. *Myosotis scorpioides* occurs in moist habitats. *Solanum dulcamara* is self-incompatible (Golas et al. 2010), *P. axillaris* (Tsukamoto et al. 1999) and *V. tricolor* (Słomka et al. 2011) are mostly outcrossing, and *L. inflata* is self-compatible (Simons and Johnston 2000). Common pollinators are bees for *V. tricolor* (Lankinen 2001), hawkmoths for *Petunia axillaris* (Hoballah et al. 2005), *Bombus* spp. for *S. dulcamara* (Liu et al. 1975), and syrphids and other flies for *M. scorpioides* (Stiers et al. 2014).

Table 1 The five focal species and plants used as neighbors in pollination experiments in South Deerfield, Massachusetts, USA, in 2013

Focal species [No. focal plants/array]	Dates of experiment	Congener	Unrelated 1	Unrelated 2
<i>Viola tricolor</i> L. (Violaceae) [6]	12–28 Jun	<i>Viola arvensis</i> L. (Violaceae)*	<i>Campanula punctata</i> x <i>C. trachelium</i> 'Viking' (Campanulaceae)	<i>Sinapis alba</i> L. (Brassicaceae)
<i>Petunia axillaris</i> (Lam.) B.S.P. (Solanaceae) [4]	10–23 Jul	<i>Petunia integrifolia</i> (Hook.) Schinz and Thellung (Solanaceae)*	<i>Silene latifolia</i> Poir (Caryophyllaceae)	<i>Centaurea cyanus</i> L. (Asteraceae)
<i>Solanum dulcamara</i> L. (Solanaceae) [2]	6–21 Aug	<i>Solanum carolinense</i> L. (Solanaceae)	<i>Veronica longifolia</i> L. (Plantaginaceae)	<i>Impatiens capensis</i> Meerb (Balsaminaceae)*
<i>Myosotis scorpioides</i> L. (Boraginaceae) [4]	11–26 Aug	None	<i>Linum usitatissimum</i> L. (Linaceae)	<i>Verbena hastata</i> L. (Verbenaceae)*
<i>Lobelia inflata</i> L. (Campanulaceae) [3]	27 Aug–13 Sept	None	<i>Cymbalaria muralis</i> P.G. Baertn., B. Mey., and Scherb. (Plantaginaceae)	<i>Malva moschata</i> L. (Malvaceae)

Each focal species was planted alone (control) or with one of three neighbor treatments (congener, unrelated 1, unrelated 2) to examine how floral similarity and phylogenetic distance affected pollinator-mediated interactions, measured by pollen limitation. Neighbor species marked with an asterisk (*) are known to co-occur with the focal species

Study site

The study was conducted between 12 June and 13 Sept 2013 at the 385-acre University of Massachusetts research farm in South Deerfield, Massachusetts, USA, in an unshaded, mowed field (42.479570° N, – 72.579248° W) that was approximately 30-x-90 m. The farm runs along the Connecticut River and is surrounded by forest and other agricultural areas. Experiments involving *Solanum dulcamara*, a species that occurs in partial shade, were conducted in a woodland edge (42.479102° N, – 72.581279° W), approximately 160 m from the field.

Experimental design

Experiments for each of the five focal species were conducted sequentially for approximately two weeks and did not overlap temporally with the exception of *S. dulcamara* and *Myosotis scorpioides* arrays, which were conducted at different locations. Each focal species was studied with two or three neighboring species (Table 1) and placed in arrays with neighbor treatments: control (no neighbor), congener, and two non-familial neighbors (hereafter referred to as “unrelated 1” and “unrelated 2” for simplicity). There was no congener treatment for two of the focal species because the intended congener did not co-flower, resulting in 13 species pairs (3 focal species * 3 neighbors + 2 focal species * 2 neighbors; Table 1) plus the control arrays. Each array was replicated five times per treatment per focal species. Arrays contained 2–6 focal plants depending on the size and availability of each focal species (Table 1). All arrays for a given focal species contained the same number of focal plants, and we attempted to standardize floral density across focal species. The number of open flowers per focal plant was later recorded during pollinator observations (Online Resource 1). Subsequent analyses found that flower density did not affect pollen limitation ($F_{1,42} = 0.07$, $P = 0.8$). Neighbor plants were added in equal numbers surrounding the focal species with one pot's width between adjacent plants so that all focal plants within an array experienced similar density and identity of neighbors. For arrays with at least three focal plants, neighboring plants were placed in a ring around focal plants, rather than in a checkerboard pattern, so that all focal plants experienced a similar environment in terms of number and identity of neighbors (Online Resource 2). Arrays with neighbors thus contained twice the plants that control arrays did. This allowed us to characterize how different neighbors affect a target group of focal plants, so we could assess whether floral trait similarity and phylogenetic relatedness mediate outcomes. However, we could not compare the effects of intraspecific versus interspecific interactions via pollinator sharing because there were more total plants in neighbor than control treatments. All arrays for each

focal species were placed simultaneously in the field 8 m apart and arranged in blocked rows with treatment randomly arranged in each block. We note that spacing between arrays could affect pollinator species differently, but other studies with multiple pollination arrays in the same field similarly ensured that the distance between arrays was greater than the array diameter (Bosch and Waser 2001; Brown et al. 2002). Because treatments were randomly arranged in blocks, it is unlikely that differences among neighbor treatments were biased by their proximity to other specific treatments. *Solanum dulcamara* arrays were arranged in a single line (only 4 m apart due to limited space) instead of a grid to be equidistant from the woodland edge; blocks were arranged linearly with treatments randomized within block.

Response variables

Pollination service to focal species was evaluated as pollinator visitation and pollen limitation. However, we observed no pollinators for 69% of our observation periods and thus had little power to detect effects of neighbor treatment on pollinator visitation. Methods and results relating to pollinator visitation, including an analysis of pollinator overlap are presented in Online Resource 1.

To measure pollen limitation, up to five flowers (or inflorescences for *Myosotis scorpioides*) on half the focal plants per array received supplemental pollen with a paintbrush, and the remaining plants were only open-pollinated. Supplemental pollen was a mixture from multiple non-experimental stock plants. We counted seeds per flower and used total seeds for up to five fruits per plant (some flowers did not set fruit) as the response variable (see ‘Data Analysis, Pollen limitation’ below). One limitation of only some flowers being hand pollinated is that plants may shift resources toward flowers that received supplemental pollen, overestimating pollen limitation (Ashman et al. 2004; Knight et al. 2006). However, it was not feasible to supplement pollen at the whole-plant level on many focal species within one season, and since many of our focal species were perennials, even whole-plant manipulations could still cause undetected resource shifts that affect pollen limitation. Many other studies of pollen limitation were similarly constrained to partial pollination (Knight et al. 2005). Furthermore, pollen supplementation of a single flower provided similar measurements of pollen limitation relative to whole-plant supplementation in *Clarkia xantiana* subsp. *parviflora* (Runquist and Moeller 2013).

Quantifying floral similarity and phylogenetic relatedness

Our goal was to assess the influences of floral similarity and phylogenetic relatedness on the outcome of interactions via

shared pollinators. We measured floral traits on up to ten non-experimental plants from each species (further details in Online Resource 2). We recorded nectar spur (present or absent), flower symmetry (radial or bilateral), and ultraviolet patterns (present or absent) by viewing flowers under ultraviolet light and observing fluorescence. For up to three flowers per plant, we measured corolla depth (the length of petal fused along the proximal–distal axis) and corolla length and width at the flower opening. For a head or a dense spike, we measured the length and width of the inflorescence instead of the individual flower. To measure nectar, we kept plants indoors for 24 h to prevent visitation, dissected one flower per plant, and collected nectar in microcapillary tubes. Because nectar production was low overall, it was calculated as the proportion of sampled flowers containing nectar. Finally, we recorded plant height for each plant in an array and then calculated mean plant height per species. We measured flower color using image analysis of scanned flowers. We first measured average RGB values of flower petals and then calculated hue, chroma, and luminance (three separate traits; Online Resource 2). Floral similarity between each species pair was calculated with a Euclidean dissimilarity matrix based on measured traits for each species. Combining traits into categories (size/shape, nectar, or color) to simplify analyses did not result in significant predictors of pollinator-mediated interaction (data not shown), and so all traits were included as separate predictors.

To assess the relationship between phylogenetic relatedness and pollinator-mediated interactions, we first inferred a phylogeny of the five focal species and thirteen neighboring species using nucleotide sequences from four loci obtained via GenBank (Benson et al. 2013; Online Resource 3). We then calculated phylogenetic distance between focal and each neighbor species using branch length data from the phylogenetic consensus tree (Online Resource 3). Patristic distances (measures of genetic change between species pairs) were calculated using branch lengths along the phylogenetic tree in R using the “cophenetic” command from the Picante package (Kembel et al. 2010).

Data analysis

All statistics were conducted in R 3.1.3 or R 3.4.0 for those using natural log offset (R Core Team 2017).

Pollen limitation

We assessed pollen limitation separately for each focal species using seed set as the response, comparing effects of neighbor treatments to the control treatment within focal species to determine which interactions were facilitative versus competitive. We also calculated pollen limitation at the array level for analyses combining all focal species to assess

whether floral similarity and phylogenetic relatedness affect the outcome of pollinator sharing (see [Floral similarity and phylogenetic distance](#) below).

We compared the effect of pollen supplementation on seed set for each focal species separately to assess pollen limitation in each neighbor treatment. Least-square means were adjusted using the function ‘lsmmeans’ from the package lsmmeans (Lenth and Hervé 2015) for array number and additional factors as necessary (see Online Resource 3 for details of model selection). We analyzed the effect of neighbor treatment, pollen supplementation, and their interaction as fixed effects on total seeds for up to five fruits per plant as the response using a generalized linear model with the ‘glmer’ function. Plant was the unit of replication, and array number (15–20 arrays per focal species; five replicate arrays per neighbor treatment plus the control) was included as a random factor. We selected a Poisson error distribution and included the natural log of the number of treated flowers per plant as an offset because some plants produced fewer than five flowers. We used flowers as an offset rather than fruits to account for flowers that did not produce fruits, potentially due to lack of pollination.

We conducted pairwise comparisons of seed set between pollen supplemented and unsupplemented plants to determine which neighbor treatments were pollen limited for each focal species. Higher seed set in pollen supplemented relative to unsupplemented plants indicated pollen limitation. If unsupplemented plants produced more seeds, then pollinators provided a greater quantity or quality of pollen than hand pollination did. A significant interaction between neighbor treatments and pollen supplementation on seed set indicates that pollen limitation differed among neighbor treatments. In those cases, we conducted a priori pairwise comparisons of the effect of pollen supplementation on seed set between each neighbor treatment and the control. Greater pollen limitation in a neighbor treatment relative to the control indicates competition, while lower pollen limitation in a neighbor compared to the control treatment indicates facilitation. All pairwise comparisons were conducted using the ‘testInteractions’ from the packagephia (De Rosario-Martinez et al. 2015) with Bonferroni adjustments.

Floral similarity and phylogenetic distance

We assessed the competition-trait similarity and competition-relatedness hypotheses via multiple regression, combining the results from all five focal species. We included both floral similarity (a single value for each focal-neighbor species pair based on the dissimilarity matrix of floral traits) and phylogenetic distance as predictors in multiple regression because multicollinearity was low (variance inflation factor = 1.1). We used pollinator-mediated interaction (competition or facilitation; see next paragraph) for each species

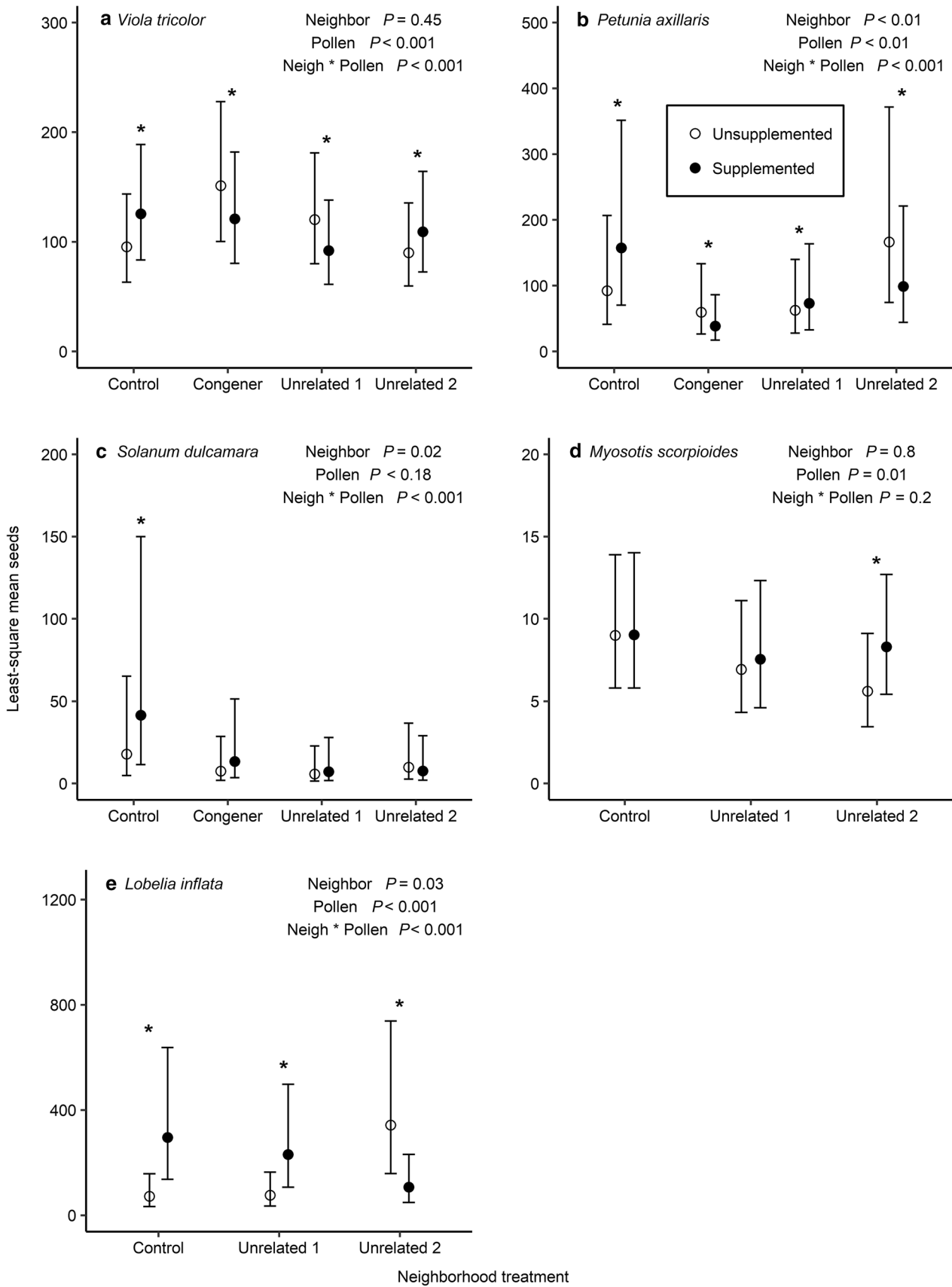


Fig. 1 Least-square mean total seeds per plant, adjusted for flower number, in five focal species with pollen-supplemented vs. unsupplemented plants. Five focal species—**a** *Viola tricolor*, **b** *Petunia axillaris*, **c** *Solanum dulcamara*, **d** *Lobelia inflata*, and **e** *Myosotis scorpioides*—were individually either grown alone (“Control”), with congeners (“Congener”), or with distantly related neighbors (“Unrelated 1” and “Unrelated 2”). Error bars show 95% confidence intervals with Bonferroni adjustments. Asterisks indicate where supplemented and unsupplemented treatments differed at $\alpha=0.05$ within neighbor treatment. Pollen limitation was greater in the control than with neighbors for all treatments in B and C, for the Congener and Unrelated 1 in A, and for Unrelated 2 in E ($\chi^2 \geq 8.1$, $d.f. = 1$, $P < 0.05$ for all). No other pairwise comparisons between neighbor treatments and the control were significant. *P*-values are reported for the effect of neighbor treatment (“Neighbor”), pollen supplementation (“Pollen”), and the interaction between the two (“Neigh * Pollen”) on total seeds

pair as the response. If species with more similar traits have more negative interactions (competition rather than facilitation), this would support the competition-trait similarity hypothesis. If more closely related species pairs have more negative interactions, this would support the competition-relatedness hypothesis.

When analyzing all focal species together, pollen limitation was standardized so it could be compared across focal species by first calculating a single value for each array (the difference between lmean seed set for supplemented and unsupplemented plants in that array) and then dividing by the overall least-square mean seed set for that focal species. Pollen limitation was then used to calculate the degree of pollinator-mediated interaction for each species pair as follows: There were five replicate arrays containing both the focal species and a neighbor species, and five replicate control arrays for each focal species. The pollen limitation of each neighbor species array was subtracted from the pollen limitation of each relevant control array to calculate 25 values (5 neighbor * 5 control arrays) indicating the degree of pollinator-mediated interaction for each species pair. There were thus 325 total pollinator-mediated interaction values (25*13) for all 13 species pairs combined.

We used multiple regression of floral similarity and phylogenetic distance on pollinator-mediated interaction to estimate parameters (slopes) but not to determine whether these parameters were significantly different than zero (calculate *P*-values). Since the 325 pollinator-mediated interaction values were not independent, we used random permutations to determine the significance of the slopes estimated in the multiple regression using the shuffleSet() function from the package permute (Simpson et al. 2014). We permuted the dependent variable (pollinator-mediated interaction) 1000 times and conducted a multiple regression on each permutation to make a null distribution of slopes. A positive slope from the original data that was above 95% of the null distribution (or a negative slope from the original data that was below 5% of the null distribution) would indicate

a significant relationship. This approach produces a similar Type I error to traditional analyses if there are no outliers and if multicollinearity between the independent variables is low (Kennedy and Cade 1996), as was the case in this study. Furthermore, results from this approach do not depend on sample size and so avoid concerns of pseudoreplication. Averaging our data to a single value for each species pair would unnecessarily eliminate variation in the data. Since each focal species was used in multiple species pairs, the thirteen species pairs were not truly independent. To account for this, we conducted permutations in two ways: (1) entirely at random (y-values were pooled across all species and randomly redistributed) and (2) within a focal species. Qualitative differences in results using these two types of permutations would suggest that species pairs including the same focal species tended to cluster together in the data cloud.

Results

Pollen limitation

All species except *M. scorpioides* were pollen-limited in control arrays (Fig. 1); in these arrays, supplemental pollination increased seed set by 37% (*V. tricolor*), 70% (*P. axillaris*), 173% (*S. dulcamara*), and 123% (*L. inflata*). Neighbors generally facilitated pollination of focal species and reduced pollen limitation (Fig. 1; Table 2). For all three congener pairs and six of the 10 unrelated species pairs, focal species were less pollen limited growing with neighbors than in the control treatment. In the other pairs, there was no significant difference in neighbor compared to control treatments (Fig. 1; Table 2), indicating neutral rather than competitive interactions.

Floral similarity and phylogenetic distance

Floral similarity decreased the extent of pollinator-mediated facilitation (no competition was observed; Fig. 2). The slope of the relationship between floral similarity and pollinator-mediated interaction ($\beta = -0.625$) was less than the 0.1 percentile of slopes from entirely random permutations ($\beta = -0.600$), which indicates a probability of 0.001 of our observed slope happening by chance. The observed slope was also less than the 1st percentile of slopes from permutations within each focal species ($\beta = -0.614$), indicating $P < 0.01$.

The slope of the relationship between phylogenetic distance and pollinator-mediated interaction ($\beta = -1.590$) was less than the 1st percentile of slopes from entirely random permutations ($\beta = -1.329$), indicating $P < 0.01$, but was greater than the 5th percentile of slopes from permutations within each focal species ($\beta = -1.961$), indicating $P > 0.05$.

Table 2 Effects of floral neighbor (neighbor treatment), pollen supplementation, and their interaction on seed set for five focal species in South Deerfield, Massachusetts, USA, in 2013

	<i>Viola tricolor</i>	<i>Petunia axillaris</i>	<i>Solanum dulcamara</i>	<i>Myosotis scorpioides</i>	<i>Lobelia inflata</i>
Neighbor treatment	2.6 (3)	12.1 (3)**	9.5 (3) *	3.1 (2)	7.2 (2)*
Pollen supplementation	84.3 (1)***	7.2 (1)**	1.8 (1)	6.1 (1)*	676.4 (1)***
Neighbor x pollen	206.4 (3)***	624.6 (3)***	33.0 (3)***	3.6 (2)	1513.4 (2)***
A priori contrasts of neighbor and control treatments					
Congener	253.3 (1)***	343.2 (1)***	20.4 (1)***	NA	NA
Unrelated 1	119.1 (1)***	43.3 (1)***	8.1 (1)*	NA	2.2 (1)
Unrelated 2	3.1 (1)	406.1 (1)***	22.4 (1)***	NA	565.8 (1)***

Cells contain Chi-square values with degrees of freedom in parentheses. The last three rows show whether pollen limitation (the difference in seed set between pollen-supplemented and unsupplemented plants) differed between the specified neighbor treatments and the control using a priori contrasts. These contrasts were not conducted for *M. scorpioides* because seed set did not differ with neighbor treatment, or for the congener treatment for *L. inflata* because this treatment was missing for this focal species. In all cases when neighbor treatments differed from the control, plants were more pollen limited in the control than the neighbor treatment (indicating facilitation; see Fig. 1). Full models included different significant factors and covariates for each focal species, as specified in Online Resource 4

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

In other words, there was a significant negative correlation between phylogenetic distance and pollinator-mediated interaction (indicating closely related species interacted more positively), but this relationship was no longer significant after controlling for focal species identity.

Discussion

The goal of this study was to determine the predictive value of phylogenetic relatedness and floral trait similarity in the outcome of pollinator-mediated interactions. We found that facilitation decreased with floral trait similarity and increased with phylogenetic relatedness (without controlling for focal species identity). These results are consistent with the competition-trait similarity hypothesis (that species with similar traits will interact more negatively); however, these results do not support the competition-relatedness hypothesis (that closely related species will interact more negatively).

Frequency of facilitation

We observed facilitation in nine of 13 species pairs and found no evidence of pollinator-mediated competition. Pollinator-mediated competition is reported more frequently in the literature (Bjerknes et al. 2007; Morales and Traveset 2009; Mitchell et al. 2009), but this does not necessarily reflect the rarity of pollinator-mediated facilitation (Feldman et al. 2004). There are multiple factors that may

contribute to the occurrence and detection of facilitation in nature. For example, facilitation may be more common at a larger spatial scale than pollinator-mediated interactions are frequently studied (Johnson et al. 2003). Experiments involving species pairs may frequently find evidence for competition if species suspected of interacting competitively are preferentially studied. Furthermore, competition is more likely to be detected when plant species are experimentally arranged in a regular, alternating pattern (interspersed; Charlebois and Sargent, 2017). Additionally, some studies may only measure pairwise pollinator-mediated interactions without considering multiple species interactions. Facilitation was common (14 of 17 interspecific pollinator-mediated interactions) in a study that simultaneously considered the effects of multiple floral neighbors in a naturally occurring plant community (Hegland et al. 2009). Facilitation may also be more common when pollinators are scarce (Tur et al. 2016), as was the case in our study. At low floral densities, the benefits of joint pollinator attraction may outweigh the potential costs of increased heterospecific pollen transfer or neighbors monopolizing pollinators (Rathcke 1983; Muñoz and Cavieres 2008).

Pollinator-mediated facilitation can occur due to changes in pollinator visitation (Hegland 2014), identity (Moeller 2005), or behavior (Liao et al. 2011) and pollen quantity (McKinney and Goodell 2011) or quality (Liao et al. 2011). Due to low pollinator visitation, we were unable to determine the mechanism behind the observed pollinator-mediated facilitation (Online Resource 1).

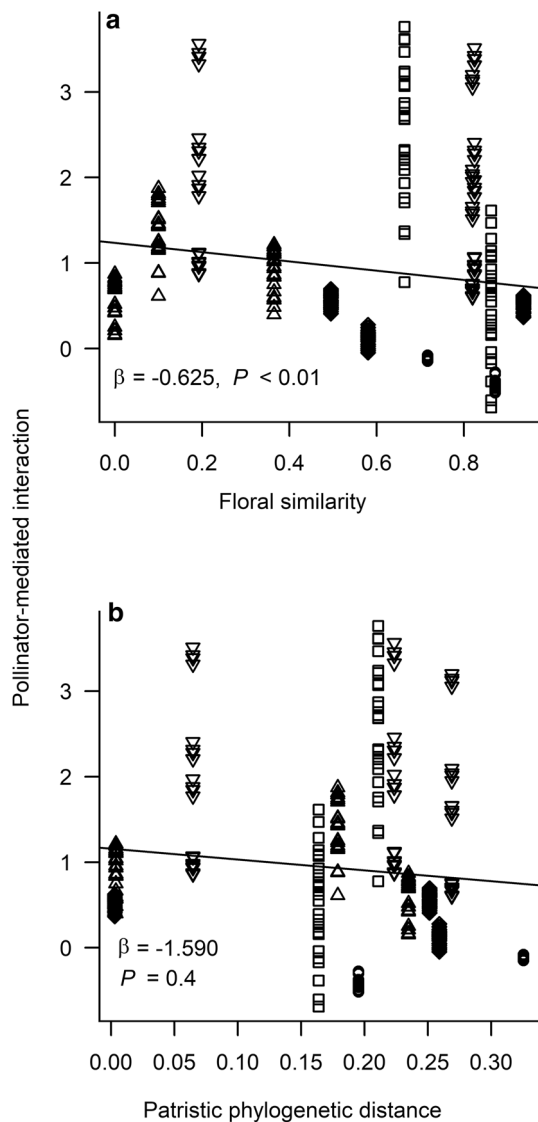


Fig. 2 The regression of pollinator-mediated interaction on **a** floral similarity, calculated from a Euclidean dissimilarity matrix of multiple floral traits, and **b** phylogenetic pairwise distance, the number of genetic differences between species. Results are from five focal species (13 species pairs). *P*-values are based on null distributions from permutations within focal species. Values greater than zero represent positive pollinator-mediated interactions (facilitation) while less than zero represent negative pollinator-mediated interactions (competition). Symbols represent the five focal species: squares (*Lobelia inflata*), circles (*Myosotis scorpioides*), triangles point up (*Petunia axillaris*), triangles point down (*Solanum dulcamara*), and diamonds (*Viola tricolor*). The slope (β) and *P*-value are based on the multiple regression and do not precisely reflect the trendline for the simple regressions shown

Competition-trait similarity hypothesis

Morphologically similar flowers experienced the least positive interactions (Fig. 2), which supports the competition-trait similarity hypothesis and is consistent with a

previous meta-analysis (Morales and Traveset 2009). Our study extends this meta-analysis, which included only flower color and symmetry as floral traits, by measuring color as a continuous variable (instead of categorical classifications) and including additional traits such as nectar volume, ultraviolet patterns, and flower size.

Species with similar floral traits may experience less positive interactions or even pollinator-mediated competition because pollinators indiscriminately move between them, increasing heterospecific pollen transfer (Morales and Traveset 2009; de Jager et al. 2011), and reducing pollination success (Campbell and Motten 1985; Bell et al. 2005). Although our limited pollinator observation data found no evidence that floral similarity decreased the proportion of conspecific pollinator movements (Online Resource 1), more extensive visitation data are needed to rigorously test this potential mechanism.

Another mechanism that could explain a negative correlation between floral similarity and pollinator-mediated interaction occurs when pollinators use plants with dissimilar flowers in complementary ways. For example, pollinator visits to *Raphanus raphanistrum*, which provided a pollen source, increased in the presence of nectar-rich species such as *Cirsium arvense* and *Hypericum perforatum* in experimental plots (Ghazoul 2006). If this mechanism explained support for the competition-trait similarity hypothesis in our study, we would expect that differences in the measured nectar traits (presence and volume) would correlate with pollinator-mediated facilitation. When we considered nectar trait similarity alone (without considering flower color, size, and shape), however, we found no such correlation (data not shown). To truly determine whether complementary floral rewards increase facilitation among dissimilar flowers in our study, we would also need to measure nectar sugar concentration as well as pollen abundance and nutritional content.

Under certain circumstances, strong facilitation may occur between species with similar floral traits. Pollinator-mediated facilitation is associated with convergent floral evolution in several systems with low floral densities; species with morphologically similar flowers in these examples are best at jointly attracting and maintaining pollinators potentially because floral similarity increases pollinator overlap (Brown and Kodric-Brown 1979; Schemske 1981). For example, floral similarity between the invasive tree *Acacia saligna* and its native floral neighbors was correlated with increased pollinator overlap (Gibson et al. 2012). Based on nonsignificant patterns in our limited pollinator observation dataset, we speculate that more extensive pollinator observations would reveal that facilitation is likely when plant species have highly overlapping pollinator communities (Online Resource 1).

Competition-relatedness hypothesis

We hypothesized that closely related species would be more likely to compete for pollinators (i.e., have a negative pollinator-mediated interaction value), but competition did not occur in our study. We also predicted that phylogenetic distance would be positively correlated with pollinator-mediated interaction. In contrast, we found a negative correlation (distantly related species facilitated each other less), although this relationship was not significant after controlling for focal species identity (Fig. 2). There was also no difference in the extent to which congeners and unrelated neighbors facilitated pollination to the focal plant (Fig. 1), indicating little support for the competition-relatedness hypothesis. This result contrasts with other studies. For example, conspecific pollen deposition to *Allium cernuum* decreased in communities of closely related floral neighbors (Schuett and Vamosi 2010). Additionally, a meta-analysis found a positive correlation between the phylogenetic distance of co-flowering neighbors and focal plant reproductive success, but this pattern held only when neighbors were alien rather than native (Morales and Traveset 2009). Two unique attributes of the present study may explain these contrasting results: (1) our study included both alien and native focal species (rather than only native focal species), and (2) our comparative approach, which involved the same experimental manipulations of multiple focal species, was unaffected by publication bias. Scientists may be less likely to publish negative results, which can lead to their underrepresentation in meta-analyses (Jennions and Møller 2002). Studies of pollinator-mediated interactions are often investigating competition, and results documenting pollinator-mediated facilitation or neutral interactions may more often be unpublished.

While closely related species are predicted to compete under the competition-relatedness hypothesis, they experienced facilitation in multiple systems. Mutualist-mediated interactions often contradict the competition-relatedness hypothesis when related species share specialist mutualists (Beltrán et al. 2012). For example, congeners facilitated pollination of *Clarkia xantiana xantiana* populations by specialist pollinators, possibly through providing staggered floral resources throughout the season (Moeller 2004). Our observations demonstrated that all study species were visited by multiple generalist pollinators except *L. inflata* (Online Resource 1), suggesting that closely related species did not have a unique opportunity for facilitation. Alternatively, if facilitative interactions between congeners often occur through joint maintenance of pollinators, our short-term study of potted experimental plants would not be sufficient to detect them. Beaudrot et al. (2013) hypothesized that character displacement in response to competition between closely related species is one possible explanation for systems that

found no evidence for the competition-relatedness hypothesis. As only four of the 13 species pairs in the present study were known to naturally co-occur, character displacement is unlikely to explain our findings. A separate study of these four co-occurring species pairs comparing allopatric and sympatric populations would be necessary to test for character displacement.

The relationship between phylogenetic distance and extent of facilitation was more negative than 99% of slopes when values were permuted fully at random ($P < 0.01$), but this relationship was not significant when the data were permuted within focal species. This suggests that the outcome of pollinator-mediated interactions was in part determined by the identity of the focal species; that is, the outcome of pollinator-mediated interactions depends on plant species identity (Ha and Ivey 2017). For example, strong facilitation (relative to the other species pairs) occurred in all the *S. dulcamara* species pairs, and neither competition nor facilitation was detected in either *M. scorpioides* species pair (as indicated by the lack of significant interaction between neighbor treatment and pollen supplementation; Table 2).

Interplay between the competition-relatedness and competition-trait similarity hypotheses

The competition-relatedness hypothesis predicts that closely related species will compete because their traits are expected to overlap (Darwin 1859; Cahill Jr. et al. 2008). If we extend this prediction to include positive interactions, there should be less facilitation between species with more similar traits. We did find that floral similarity reduced facilitative interactions (Fig. 1). There was little multicollinearity between phylogenetic distance and floral similarity, however, indicating that the flowers of closely related species do not necessarily resemble each other as previously suggested (Memmott and Waser 2002; Morales and Traveset 2009), at least in the traits we measured (flower size, shape, color, and nectar production). Other studies are consistent with our results, finding no correlation between floral trait similarity and phylogenetic relatedness (de Jager et al. 2011; Bergamo et al. 2018). Thus, relationships between phylogenetic relatedness and species interactions may be mediated by other traits not considered in this study, such as floral volatiles, nectar sugar concentration, pollen production, or diameter of corolla tube opening.

Caveats

There are several limitations of our results. First, we focused on the outcome of species interactions at a small scale, with 8 m between most arrays, and the outcome of pollinator-mediated interactions may differ with spatial scale (Braun and Lortie 2019). For example, co-flowering

plant species may facilitate pollination at large scales by attracting pollinators to the general area but may compete at a local scale by drawing pollinators away from one another (Jakobsson et al. 2009). However, even at our small scale we found more evidence of facilitation than competition.

Second, our neighbor treatments increased the total size and floral display of the arrays because the number of focal plants was held constant. When we concluded that a neighbor facilitated a focal species, we could not compare this with intraspecific pollinator-mediated interactions that could occur at higher conspecific densities. A study of a temperate grassland community found both intraspecific and interspecific pollinator-mediated facilitation. Flower visitation rate to *Campanula rotundifolia* increased with both conspecific floral density and with *Euphrasia stricta* and *Knautia arvensis* floral density (Hegland et al. 2009). It is possible that conspecifics could provide greater facilitation than heterospecifics because they would not cause heterospecific pollen transfer. Nevertheless, our results highlight the importance of interspecific pollinator-mediated facilitation in cases where focal species occur at low densities.

Third, facilitation in *S. dulcamara* and *P. axillaris* was driven by particularly high seed set in pollen-supplemented plants in the control treatment (Fig. 1). Neighbor treatments were randomly placed within blocks, so they should not experience consistently different microenvironments. Furthermore, if this were the case, we would expect both pollen-supplemented and unsupplemented seed set to be affected. While there is no obvious explanation for this pattern, it is possible that control plants experienced reduced competition for light because there were half as many plants in the control compared to neighbor treatments. If access to light was greater in the control treatment, control plants could have had more resources to increase seed set when pollen supplemented relative to plants with neighbors.

Fourth, in some cases unsupplemented plants had higher seed set than pollen-supplemented plants (Fig. 1). This pattern has occurred in several other studies (González-Varo and Traveset 2010; Razanajatovo and Kleunen 2016) and indicates that pollinators provided higher quantity or quality of pollen than hand pollination. Pollinators could deposit pollen that had just been removed from a donor, but hand pollination relied on pollen collected up to three hours earlier. However, the order of hand pollination was randomized across arrays so that no treatment would get consistently older pollen. Unsupplemented plants produced more seeds than pollen supplemented ones with remarkable consistency within a neighbor treatment, occurring in all five replicates or not at all. Such consistency within treatments suggests a biological explanation rather than methodological errors, such as higher quality of pollen delivered by the pollinator community that visited arrays with particular neighbors.

Conclusion and application

This is the first manipulative study to examine the conditions that determine pollinator-mediated interactions across plant species simultaneously. We found that pollinator-mediated facilitation decreased with floral trait similarity and phylogenetic distance. The combination of our data and other studies on floral traits, phylogenetic relatedness, and factors such as relative species abundance (e.g., Runquist and Stanton 2013; Bruckman and Campbell 2016), spatial arrangement (Bruckman and Campbell 2016), and spatial scale (e.g., Johnson et al. 2003; Cariveau and Norton 2009) could build a powerful model that predicts the outcome of pollinator sharing between species. Considering how neighboring plant species interact could improve habitat management of rare species by suggesting which species promote facilitation. Similarly, management of invasive plants may involve supporting native plant populations that strongly compete for pollinators. Understanding the factors that promote facilitation versus competition in ecosystem has important potential applications as well as improving our ability to predict how species interact in communities.

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Author contribution statement MKH conceived of and designed the study with guidance from LSA. MKH conducted the study. SAS conducted all analyses pertaining to calculating phylogenetic distance. MKH conducted all other data analysis with input from LSA. MKH wrote the study with guidance from LSA, and all authors provided feedback on the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Arceo-Gómez G, Ashman T-L (2016) Invasion status and phylogenetic relatedness predict cost of heterospecific pollen receipt: implications for native biodiversity decline. *J Ecol* 104:1003–1008. <https://doi.org/10.1111/1365-2745.12586>
- Ashman T-L, Knight TM, Steets JA et al (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85:2408–2421. <https://doi.org/10.1890/03-8024>
- Beaudrot L, Struebig MJ, Meijaard E et al (2013) Co-occurrence patterns of Bornean vertebrates suggest competitive exclusion is strongest among distantly related species. *Oecologia* 173:1053–1062. <https://doi.org/10.1007/s00442-013-2679-7>
- Bell JM, Karron JD, Mitchell RJ (2005) Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology* 86:762–771. <https://doi.org/10.2307/3450670>
- Beltrán E, Valiente-Banuet A, Verdú M (2012) Trait divergence and indirect interactions allow facilitation of congeneric species. *Ann Bot* 110:1369–1376. <https://doi.org/10.1093/aob/mcs089>
- Benson DA, Cavanaugh M, Clark K et al (2013) GenBank. *Nucleic Acids Res* 41:D36–D42. <https://doi.org/10.1093/nar/gks1195>
- Bergamo PJ, Wolowski M, Maruyama PK et al (2018) Trait patterns across space and time suggest an interplay of facilitation and competition acting on Neotropical hummingbird-pollinated plant communities. *Oikos* 127:1690–1700. <https://doi.org/10.1111/oik.05571>
- Bjerknes A-L, Totland Ø, Hegland SJ, Nielsen A (2007) Do alien plant invasions really affect pollination success in native plant species? *Biol Conserv* 138:1–12. <https://doi.org/10.1016/j.biocn.2007.04.015>
- Bosch M, Waser NM (2001) Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia* 126:76–83. <https://doi.org/10.1007/s004420000488>
- Braun J, Lortie CJ (2019) Finding the bees knees: a conceptual framework and systematic review of the mechanisms of pollinator-mediated facilitation. *Perspect Plant Ecol Evol Syst* 36:33–40. <https://doi.org/10.1016/j.ppees.2018.12.003>
- Brooker RW, Callaway RM (2009) Facilitation in the conceptual melting pot. *J Ecol* 97:1117–1120. <https://doi.org/10.1111/j.1365-2745.2009.01580.x>
- Brooker RW, Maestre FT, Callaway RM et al (2008) Facilitation in plant communities: the past, the present, and the future. *J Ecol* 96:18–34. <https://doi.org/10.1111/j.1365-2745.2007.01295.x>
- Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology* 83:2328–2336. [https://doi.org/10.1890/0012-9658\(2002\)083\[2328:CFPBAI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2328:CFPBAI]2.0.CO;2)
- Brown JH, Kodric-Brown A (1979) Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* 60:1022–1035. <https://doi.org/10.2307/1936870>
- Bruckman D, Campbell DR (2016) Pollination of a native plant changes with distance and density of invasive plants in a simulated biological invasion. *Am J Bot* 103:1458–1465. <https://doi.org/10.3732/ajb.1600153>
- Cahill JF Jr, Kembel SW, Lamb EG, Keddy PA (2008) Does phylogenetic relatedness influence the strength of competition among vascular plants? *Perspect Plant Ecol Evol Syst* 10:41–50. <https://doi.org/10.1016/j.ppees.2007.10.001>
- Cameron DD, White A, Antonovics J (2009) Parasite–grass–forb interactions and rock–paper–scissor dynamics: predicting the effects of the parasitic plant *Rhinanthus minor* on host plant communities. *J Ecol* 97:1311–1319. <https://doi.org/10.1111/j.1365-2745.2009.01568.x>
- Campbell DR, Motten AF (1985) The mechanism of competition for pollination between two forest herbs. *Ecology* 66:554–563. <https://doi.org/10.2307/1940404>
- Cariveau DP, Norton AP (2009) Spatially contingent interactions between an exotic and native plant mediated through flower visitors. *Oikos* 118:107–114. <https://doi.org/10.1111/j.1600-0706.2008.16705.x>
- Carvalho LG, Biesmeijer JC, Benadi G et al (2014) The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. *Ecol Lett* 17:1389–1399. <https://doi.org/10.1111/ele.12342>
- Chamberlain SA, Bronstein JL, Rudgers JA (2014) How context dependent are species interactions? *Ecol Lett* 17:881–890. <https://doi.org/10.1111/ele.12279>
- Charlebois JA, Sargent RD (2017) No consistent pollinator-mediated impacts of alien plants on natives. *Ecol Lett* 20:1479–1490. <https://doi.org/10.1111/ele.12831>
- Darwin CR (1859) On the origin of species by means of natural selection. Murray, London
- de Jager ML, Dreyer LL, Ellis AG (2011) Do pollinators influence the assembly of flower colours within plant communities? *Oecologia* 166:543–553. <https://doi.org/10.1007/s00442-010-1879-7>
- De Rosario-Martinez H, Fox J, R Core Team (2015) phia: Post-hoc interaction analysis, R package version 0.2-0. <https://github.com/heliosdrm/phia>
- Dietzsch A, Stanley D, Stout J (2011) Relative abundance of an invasive alien plant affects native pollination processes. *Oecologia* 167:469–479. <https://doi.org/10.1007/s00442-011-1987-z>
- Feldman TS, Morris WF, Wilson WG (2004) When can two plant species facilitate each other's pollination? *Oikos* 105:197–207. <https://doi.org/10.1111/j.0030-1299.2004.12845.x>
- Gegeer RJ, Laverty TM (2005) Flower constancy in bumblebees: a test of the trait variability hypothesis. *Anim Behav* 69:939–949. <https://doi.org/10.1016/j.anbehav.2004.06.029>
- Ghazoul J (2006) Floral diversity and the facilitation of pollination. *J Ecol* 94:295–304. <https://doi.org/10.1111/j.1365-2745.2006.01098.x>
- Gibson MR, Richardson DM, Pauw A (2012) Can floral traits predict an invasive plant's impact on native plant–pollinator communities? *J Ecol* 100:1216–1223. <https://doi.org/10.1111/j.1365-2745.2012.02004.x>
- Golas TM, Feron RMC, van den Berg RG et al (2010) Genetic structure of European accessions of *Solanum dulcamara* L. (Solanaceae). *Plant Syst Evol* 285:103–110. <https://doi.org/10.1007/s00606-009-0260-y>
- González-Varo JP, Traveset A (2010) Among-individual variation in pollen limitation and inbreeding depression in a mixed-mating shrub. *Ann Bot* 106:999–1008. <https://doi.org/10.1093/aob/mcq200>
- Ha MK, Ivey CT (2017) Pollinator-mediated interactions in experimental arrays vary with neighbor identity. *Am J Bot* 104:252–260. <https://doi.org/10.3732/ajb.1600293>
- Harrington TB, Dagley CM, Edwards MB (2003) Above- and below-ground competition from longleaf pine plantations limits performance of reintroduced herbaceous species. *For Sci* 49:681–695. <https://doi.org/10.1093/forestscience/49.5.681>
- Hegland SJ (2014) Floral neighbourhood effects on pollination success in red clover are scale-dependent. *Funct Ecol* 28:561–568. <https://doi.org/10.1111/1365-2435.12223>
- Hegland SJ, Grytnes J-A, Totland Ø (2009) The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecol Res* 24:929–936. <https://doi.org/10.1007/s11284-008-0572-3>
- Hoballah ME, Stuurman J, Turlings TCJ et al (2005) The composition and timing of flower odour emission by wild *Petunia axillaris* coincide with the antennal perception and nocturnal activity of

- the pollinator *Manduca sexta*. *Planta* 222:141–150. <https://doi.org/10.1007/s00425-005-1506-8>
- Jakobsson A, Padrón B, Traveset A (2009) Competition for pollinators between invasive and native plants: effects of spatial scale of investigation (note). *Ecoscience* 16:138–141. <https://doi.org/10.2980/16-1-3193>
- Jennions MD, Møller AP (2002) Publication bias in ecology and evolution: an empirical assessment using the “trim and fill” method. *Biol Rev* 77:211–222. <https://doi.org/10.1017/s1464793101005875>
- Johnson SD, Peter CI, Nilsson A, Ågren J (2003) Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84:2919–2927. <https://doi.org/10.1890/02-0471>
- Kembel SW, Cowan PD, Helmus MR et al (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
- Kennedy PE, Cade BS (1996) Randomization tests for multiple regression. *Commun Stat Simul Comput* 25:923–936. <https://doi.org/10.1080/03610919608813350>
- Knight TM, Steets JA, Ashman T-L (2006) A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *Am J Bot* 93:271–277. <https://doi.org/10.3732/ajb.93.2.271>
- Knight TM, Steets JA, Vamosi JC et al (2005) Pollen limitation of plant reproduction: Pattern and process. *Annu Rev Ecol Evol Syst* 36:467–497. <https://doi.org/10.1146/annurev.ecolsys.36.102403.115320>
- Koepfel AF, Wu M (2014) Species matter: the role of competition in the assembly of congeneric bacteria. *ISME J* 8:531–540. <https://doi.org/10.1038/ismej.2013.180>
- Kunstler G, Lavergne S, Courbaud B et al (2012) Competitive interactions between forest trees are driven by species’ trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. *Ecol Lett* 15:831–840. <https://doi.org/10.1111/j.1461-0248.2012.01803.x>
- Lankinen Å (2001) In vitro pollen competitive ability in *Viola tricolor*: temperature and pollen donor effects. *Oecologia* 128:492–498. <https://doi.org/10.1007/s004420100681>
- Lenth RV, Hervé M (2015) lsmeans: Least-squares means. R package version 2.19. <https://cran.r-project.org/web/packages/lsmeans/index.html>
- Liao K, Gituru RW, Guo Y-H, Wang Q-F (2011) The presence of co-flowering species facilitates reproductive success of *Pedicularis monbeigiana* (Orobanchaceae) through variation in bumble-bee foraging behaviour. *Ann Bot* 108:877–884. <https://doi.org/10.1093/aob/mcr216>
- Liu HJ, Macfarlane RP, Pengelly DH (1975) Relationships between flowering plants and four species of *Bombus* (Hymenoptera: Apidae) in southern Ontario. *Can Entomol* 107:577–588. <https://doi.org/10.4039/Ent107577-6>
- Lortie CJ, Callaway RM (2009) David and Goliath: comparative use of facilitation and competition studies in the plant ecology literature. *Web Ecol* 9:54–57. <https://doi.org/10.5194/we-9-54-2009>
- Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecol Lett* 11:995–1003. <https://doi.org/10.1111/j.1461-0248.2008.01229.x>
- MacArthur R (1958) Population ecology of some warblers of North-eastern coniferous forests. *Ecology* 39:599–619. <https://doi.org/10.2307/1931600>
- McIntire EJB, Fajardo A (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytol* 201:403–416. <https://doi.org/10.1111/nph.12478>
- McKinney AM, Goodell K (2011) Plant–pollinator interactions between an invasive and native plant vary between sites with different flowering phenology. *Plant Ecol* 212:1025–1035. <https://doi.org/10.1007/s11258-010-9882-y>
- Memmott J, Waser NM (2002) Integration of alien plants into a native flower–pollinator visitation web. *Proc R Soc Lond B Biol Sci* 269:2395–2399. <https://doi.org/10.1098/rspb.2002.2174>
- Michalet R, Maalouf J-P, Choler P et al (2015) Competition, facilitation and environmental severity shape the relationship between local and regional species richness in plant communities. *Ecography* 38:335–345. <https://doi.org/10.1111/ecog.01106>
- Michalet R, Xiao S, Touzard B et al (2011) Phenotypic variation in nurse traits and community feedbacks define an alpine community. *Ecol Lett* 14:433–443. <https://doi.org/10.1111/j.1461-0248.2011.01605.x>
- Mitchell RJ, Flanagan RJ, Brown BJ et al (2009) New frontiers in competition for pollination. *Ann Bot* 103:1403–1413. <https://doi.org/10.1093/aob/mcp062>
- Moeller DA (2004) Facilitation interactions among plants via shared pollinators. *Ecology* 85:3289–3301. <https://doi.org/10.1890/03-0810>
- Moeller DA (2005) Pollinator community structure and sources of spatial variation in plant–pollinator interactions in *Clarkia xantiana* ssp. *xantiana*. *Oecologia* 142:28–37
- Morales CL, Traveset A (2009) A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecol Lett* 12:716–728. <https://doi.org/10.1111/j.1461-0248.2009.01319.x>
- Muchhala N, Johnsen S, Smith SD (2014) Competition for hummingbird pollination shapes flower color variation in Andean Solanaceae. *Evolution* 68:2275–2286. <https://doi.org/10.1111/evo.12441>
- Muhamed H, Touzard B, Le Bagousse-Pinguet Y, Michalet R (2013) The role of biotic interactions for the early establishment of oak seedlings in coastal dune forest communities. *For Ecol Manag* 297:67–74. <https://doi.org/10.1016/j.foreco.2013.02.023>
- Muñoz AA, Cavieres LA (2008) The presence of a showy invasive plant disrupts pollinator service and reproductive output in native alpine species only at high densities. *J Ecol* 96:459–467. <https://doi.org/10.1111/j.1365-2745.2008.01361.x>
- R Core Team (2017) R: A language and environment for statistical computing. Version 3.4.0. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rathcke B (1983) Competition and facilitation among plants for pollination. *Pollination biology*. Academic Press, New York, pp 305–329
- Razanajatovo M, van Kleunen M (2016) Non-invasive naturalized alien plants were not more pollen-limited than invasive aliens and natives in a common garden. *Funct Ecol* 30:1511–1520. <https://doi.org/10.1111/1365-2435.12633>
- Runquist RB, Stanton ML (2013) Asymmetric and frequency-dependent pollinator-mediated interactions may influence competitive displacement in two vernal pool plants. *Ecol Lett* 16:183–190. <https://doi.org/10.1111/ele.12026>
- Runquist RDB, Moeller DA (2013) Resource reallocation does not influence estimates of pollen limitation or reproductive assurance in *Clarkia xantiana* subsp. *parviflora* (Onagraceae). *Am J Bot* 100:1916–1921. <https://doi.org/10.3732/ajb.1300050>
- Schemske DW (1981) Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62:946–954. <https://doi.org/10.2307/1936993>
- Schuett EM, Vamosi JC (2010) Phylogenetic community context influences pollen delivery to *Allium cernuum*. *Evol Biol* 37:19–28. <https://doi.org/10.1007/s11692-010-9082-7>
- Simons AM, Johnston MO (2000) Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *Am J Bot* 87:124–132. <https://doi.org/10.2307/2656690>

- Simpson GL, R Core Team, Bates DM, Oksanen J (2014) permute: Functions for generating restricted permutations of data. R package version 0.8–3. <https://vegan.r-forge.r-project.org/>
- Słomka A, Sutkowska A, Szczepaniak M et al (2011) Increased genetic diversity of *Viola tricolor* L. (Violaceae) in metal-polluted environments. *Chemosphere* 83:435–442. <https://doi.org/10.1016/j.chemosphere.2010.12.081>
- Soliveres S, Smit C, Maestre FT (2015) Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. *Biol Rev* 90:297–313. <https://doi.org/10.1111/brv.12110>
- Stachowicz JJ (2001) Mutualism, facilitation, and the structure of ecological communities. *Bioscience* 51:235–246. [https://doi.org/10.1641/0006-3568\(2001\)051\[0235:MFATSO\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2001)051[0235:MFATSO]2.0.CO;2)
- Stiers I, Coussement K, Triest L (2014) The invasive aquatic plant *Ludwigia grandiflora* affects pollinator visitants to a native plant at high abundances. *Aquat Invasions* 9:357–367
- Tsukamoto T, Ando T, Kokubun H et al (1999) Breakdown of self-incompatibility in a natural population of *Petunia axillaris* (Solanaceae) in Uruguay containing both self-incompatible and self-compatible plants. *Sex Plant Reprod* 12:6–13. <https://doi.org/10.1007/s004970050166>
- Tur C, Sáez A, Traveset A, Aizen MA (2016) Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: Evidence of widespread facilitation in south Andean plant communities. *Ecol Lett* 19:576–586. <https://doi.org/10.1111/ele.12594>
- Violle C, Nemergut DR, Pu Z, Jiang L (2011) Phylogenetic limiting similarity and competitive exclusion. *Ecol Lett* 14:782–787. <https://doi.org/10.1111/j.1461-0248.2011.01644.x>
- Wagg C, Ebeling A, Roscher C et al (2017) Functional trait dissimilarity drives both species complementarity and competitive disparity. *Funct Ecol* 31:2320–2329. <https://doi.org/10.1111/1365-2435.12945>
- Webb CO, Gilbert GS, Donoghue MJ (2006) Phylodiversity-dependent seedling mortality, size structure, and disease in a Bornean rain forest. *Ecology* 87:S123–S131. [https://doi.org/10.1890/0012-9658\(2006\)87\[123:PSMSSA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[123:PSMSSA]2.0.CO;2)